BIOMÉRIEUX

TORQUE TENO VIRUS (TTV) BIOMARKER GUIDING TRANSPLANT RISK MANAGEMENT

Selection of publications

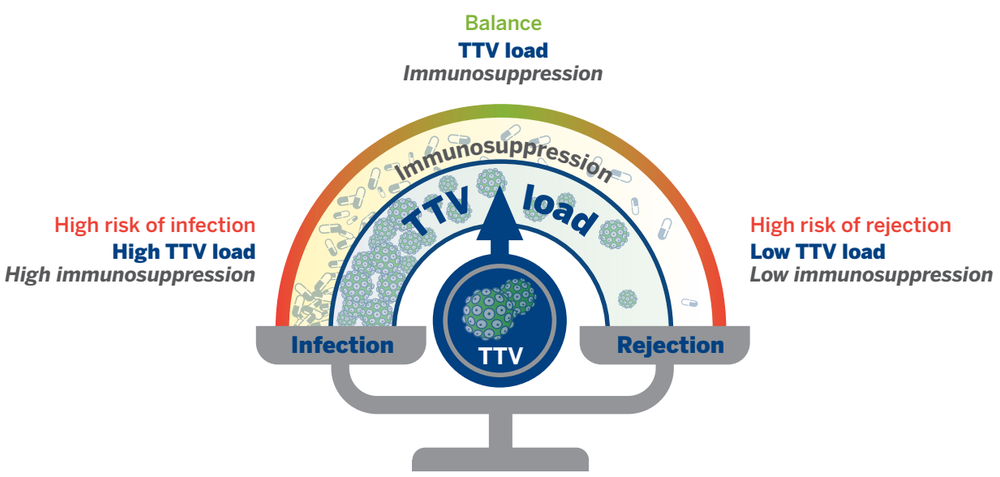
2022 EDITION

PIONEERING DIAGNOSTICS

In 2018, an estimated 146 840 solid organs were transplanted worldwide.[1]

"...despite a consistent improvement in kidney graft survival in the first 5 years post-transplant between 1986 and 2015, graft survival after the fifth year of transplantation has not substantially changed over time."[2]

"Monitoring markers of immunosuppression can (...) help to individualize immunosuppressive therapy to maximize drug efficacy and minimize toxicity."[2]



Original illustration by Joris I. Rotmans and Manon Zuurmond, Department of Internal Medicine, LUMC, Leiden. Used with permission.

Our special thanks go to Associate Professor Gregor Bond for his valuable advice and comprehensive review of this Selection of Publications.

For references, see end of document

INTRODUCTION

Allograft transplantation is the definitive treatment for end-stage solid organ dysfunction including kidney, liver, pancreas, intestine, lung and heart disease. There is a shortage in suitable donor organs leading to high numbers of patients on the waiting lists with over 58.000 patients registered in Europe.[2] Unfortunately, the overall waitlist mortality is persistently high and 21 patients die every day while waiting for an organ transplant in Europe.[3]

Besides approaches to advancing donation, long-term preservation of graft function is crucial to shorten waiting lists. In this respect, it is important to note that graft rejection due to insufficient immunosuppression is a leading cause of transplant dysfunction and loss.[4] The effect of excessive immunosuppression is equally troublesome for solid organ transplant recipients: the risk for infectious and oncologic disease is increased accounting for the most common causes of death.[5] Therefore, **optimisation of the immunosuppressive regimen to simultaneously reduce rejection, infection and oncologic disease is crucial to prolong patient and graft survival.** To date, no reliable tool to guide dosing of immunosuppressive drugs exists.

The ideal marker for the guidance of immunosuppressive drugs would simultaneously predict the consequences of both over- and under-immunosuppression. Monitoring **Torque Teno Virus (TTV)** in the peripheral blood **is a promising novel strategy used to characterise the immune function.[6]** TTV can be detected in up to 90% of healthy individuals and has not been linked to any human disease. The prevalence of TTV in immuno-compromised patients after solid organ transplantation is up to 100% and the virus is unaffected by conventional anti-viral drug therapies used in the post-transplant setting. TTV copy number is directly associated with the amount and type of immunosuppressive drugs administered to transplant recipients and additional major factors determining the immune function of its host (e.g., age and sex); thus **TTV load is indirectly associated with graft rejection and infectious disease.[7,8]**

Non-interventional studies in kidney and lung transplant recipients have identified TTV plasma load cut-off values for risk stratification of graft rejection and infectious disease and defined an optimal TTV load for guidance of immunosuppression. Based on the proposed cut-offs, two multinational, interventional, randomised controlled trials are investigating the value of TTV-guided immunosuppression in kidney and lung transplant recipients.[9,10]

TTV-based personalization and optimization of immunosuppressive drug dosing might enable clinicians to reduce infections and graft rejection in solid organ transplant recipients and thus prolong patient and graft survival and shorten transplant waitlists. Standardized assay systems with high intra- and inter-center comparability are an important pre-requisite for implementation of TTV quantification in routine clinical post-transplant care.

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For references, see end of document

CONTENTS

KIDNEY TRANSPLANTATION

LUNG TRANSPLANTATION

LIVER TRANSPLANTATION

REFERENCES

KIDNEY TRANSPLANTATION

CLICK TO ACCESS ARTICLE

AMERICAN JOURNAL OF TRANSPLANTATION
2020 AUG;20(8):2081-2090. DOI: 10.1111/AJT.15810. EPUB 2020 MAR 8

Torque Teno Virus for Risk Stratification of Graft Rejection and Infection in Kidney Transplant Recipients – A Prospective Observational Trial

K. Doberer, M. Schiemann, R. Strassl R, F. Haupenthal, F. Dermuth, I. Görzer, F. Eskandary, R. Reindl-Schwaighofer, Z. Kikić, E. Puchhammer-Stöckl, G.A. Böhmig, G. Bond

OBJECTIVES

Test for an association between TTV load and infection and graft rejection, respectively, in the first year after kidney transplantation.
Define a cut-off for an optimal TTV load range to support the investigation of TTV-guided immunosuppression during a randomized controlled interventional trial.

MATERIAL & METHODS

Prospective observational single center trial on 386 consecutive adult recipients of a kidney allograft: 274 patients were analyzed after applying inclusion and exclusion criteria.
TTV was measured (in-house PCR) at the outpatient clinic on month 3 post transplantation and every 3 months thereafter.
Primary endpoint: graft rejection determined by biopsy using BANFF classification.
Secondary endpoint: infectious events (bacterial, fungal or viral) requiring antimicrobial or antiviral treatment, reduction of immunosuppressive drugs, hospitalization or increase of hospital stay.
TTV measurements taken after TTV load stabilization at the end of post-transplant month 3 were analyzed in the context of subsequent infection and graft rejection in the first year post transplantation.

RESULTS

In the first year of transplantation:

- All but two patients had a detectable TTV infection.
- Patient survival was 96% and death censored graft survival was 95%.
- Graft rejection was diagnosed in 18% of the recipients.
- A total of 54% of the recipients experienced an infection and a total of 472 episodes of infection were detected.

Concerning rejection risk evaluation:

- Lower TTV loads in patients developing rejection were quantified in comparison to patients with no rejection.
- TTV measurement preceded subsequent biopsies by a median of 14 days.
- The odds for the development of a rejection were lowered by 22% for each log increase in TTV load (95% CI 0.62-0.97).
- A TTV load cut-off of 1.5×10^6 copies/mL predicts rejection with 89% specificity, 36% sensitivity, a negative predictive value (NPV) of 77% and a positive predictive value (PPV) of 50%.

Concerning infection risk evaluation:

- Higher TTV plasma loads in patients developing an infection were quantified in comparison to patients with no infection.
- The odds for an infection increased by 11% for each log increase in TTV load (CI 1.06-1.15).
- TTV measurement preceded subsequent biopsies by a median of 27 days.
- TTV loads above $5.8\text{x}10^9$ copies/mL predict infection with 90% specificity, 18% sensitivity, a NPV of 77% and a PPV of 37%.
  For TTV loads up to 10^9 copies/mL high NPVs were calculated for the detection of infections (range 100% to 79%) and the highest PPV was calculated for TTV loads above 10^{10} copies/mL (range 44% to 67%).

" Taken together, the results of our study provide evidence for the value of TTV quantification for risk stratification of clinically relevant graft rejection and infection after kidney transplantation. Moreover, we defined an optimal TTV range as a basis for an interventional trial to test the efficacy of TTV-guided immunosuppression reducing infection and rejection after kidney transplantation."

KEY FINDINGS

- Potential of TTV load for risk stratification of kidney graft rejection.
- Potential of TTV load for risk stratification of infection.
- Risk for the development of rejection below 10^6 copies/mL TTV load and risk for infections above 10^8 copies/mL TTV load in month 4 to 12 post kidney transplantation; thus providing a potential TTV target range to guide immunosuppressive therapy.

CLICK TO ACCESS ARTICLE

AMERICAN JOURNAL OF TRANSPLANTATION
2019 APR;19(4):1139-1149. DOI: 10.1111/AJT.15145

Monitoring of Alphatorquevirus DNA Levels for the Prediction of Immunosuppression-related Complications after Kidney Transplantation

M. Fernández-Ruiz, E. Albert, E. Giménez, T. Ruiz-Merlo, P. Parra, F. López-Medrano, R. San Juan, N. Polanco, A. Andrés, D. Navarro, J.M. Aguado

OBJECTIVE

Evaluate the potential of *Alphatorquevirus* (TTV) as a predictive factor of infection and more largely immune-related adverse events (iRAEs, which include opportunistic infections and *de novo* malignancies) occurring after kidney transplantation.

MATERIAL & METHODS

Prospective and observational study on 221 kidney transplant recipients followed-up for at least 12 months. Seven patients were excluded after graft loss or death within the 12 first months of follow-up.
Opportunistic infection was defined as due to bacterial infection (mycobacteria, *Nocardia* spp. and *Listeria monocytogenes*), CMV, HSV, VZV, biopsy-proven BK-Virus associated nephropathy (BK-VAN), yeast (*Candida* spp., *Cryptococcus* spp.), molds (invasive aspergillosis and mucormycosis) and parasites (*Toxoplasma gondii, Pneumocystis jirovecii* and *Leishmania* spp.).
TTV measurements were done on plasma (TTV R-GENE®) within 6h pre-transplantation, on day 7 (D7) and months 1, 3 , 6 and 12.
Primary endpoint: occurrence of serious infection leading to hospitalization or intravenous antimicrobial therapy and immunosuppression-related adverse events (iRAEs) and post-transplant *de novo* malignancy.

RESULTS

Infectious diseases occurred in 128 patients with 287 episodes (median time prior to 1st infection episode: 37 days (Interquartile Range (IQR):14-99.3)).
iRAE occurred in 51 patients with 65 episodes (median time prior to 1st iRAE episode: 78 days (IQR:39-235)).
Induction therapy with anti-thymocyte globulin (ATG) was associated with higher plasma *Alphatorquevirus* during the first 6 months post-transplant with significant differences at month 3 (5.5 ± 1.8 $\log_{10}$ copies/mL vs 4.8 ± 1.9 $\log_{10}$ copies/mL; $P = 0.018$):

- CD3+ at month 1 ($r = -0.238$; $P = 0.017$) and month 3 ($r = -0.347$; $P < 0.0001$)
- CD4+ at month 1 ($r = -0.241$; $P = 0.015$) and month 3 ($r = -0.330$; $P < 0.001$)
- CD8+ T cell at month 1 ($r = -0.240$; $P = 0.016$)

Dependence of TTV load and event occurrence:

- Dependence at month 1 for infection event (4.6 ± 1.3 vs. 3.8 ± 1.9 $\log_{10}$ copies/mL; $P=0.023$)
- Dependence for iRAEs at:
  - month 1 (4.9 ± 1.2 $\log_{10}$ copies/mL vs. 3.9 ± 1.8 $\log_{10}$ copies/mL; $P=0.009$)
  - month 3 (5.6 ± 1.3 $\log_{10}$ copies/mL vs 4.7 ± 1.8 $\log_{10}$ copies/mL; $P=0.007$)
  - month 6 (6.8 ± 2.0 $\log_{10}$ copies/mL vs 5.8 ± 1.7 $\log_{10}$ copies/mL; $P=0.012$)

Correlation between the cumulative magnitude of TTV viral load, estimated through the area under the curve (AUC) for $\log_{10}$ TTV load in plasma:

- Among patients with post-transplant infection, AUC were significantly higher at month 1 ($AUC_{0\text{-}30}$ 5.1 ± 1.7 $\log_{10}$ copies/mL vs 4.6 ± 1.7 $\log_{10}$ copies/mL; $P = 0.046$) and month 6 ($AUC_{0\text{-}180}$ 8.8 ± 1.3 $\log_{10}$ copies/mL vs 7.9 ± 1.6 $\log_{10}$ copies/mL; $P = 0.032$))
- Among patients with post-transplant iRAEs, AUC were significantly higher at month 1 ($AUC_{0\text{-}30}$ 5.4 ± 1.4 $\log_{10}$ copies/mL vs 4.7 ± 1.7 $\log_{10}$ copies/mL; $P = 0.015$) and month 6 ($AUC_{0\text{-}180}$ 9.1 ± 1.2 $\log_{10}$ copies/mL vs 7.9 ± 1.5 $\log_{10}$ copies/mL; $P = 0.023$)

Increase in TTV levels between D7 and M1 shows:

- A significant correlation with post-transplant infection occurrence (57.3% vs 18.8%; $P=0.005$)
- A non-significant trend with post-transplant iRAEs occurrence (26.8% vs 6.2%; $P=0.108$).

"Alphatorquevirus viremia is emerging as a feasible, comprehensive surrogate biomarker for the overall state of immunosuppression after SOT"

KEY FINDINGS

- Definition of cut-offs at 1 month post transplantation to detect patients at low-risk of developing infections and iRAEs.
- Plasma TTV kinetics might be useful to predict the occurrence of infections, but also more generally complications due to over-immunosuppression.

CLICK TO ACCESS ARTICLE

JOURNAL OF INFECTIOUS DISEASES
2019 MAY 24;219(12):1934-1939. DOI: 10.1093/INFDIS/JIZ039

Torque Teno Virus for Risk Stratification of Acute Biopsy-Proven Alloreactivity in Kidney Transplant Recipients

R. Strassl, K. Doberer, S. Rasoul-Rockenschaub, H. Herkner, I. Görzer, J. P. Kläger, R. Schmidt, H. Haslacher, M. Schiemann, F. A. Eskandary, Ž. Kikić, R. Reindl-Schwaighofer, E. Puchhammer-Stöckl, G. A. Böhmig, G. Bond

OBJECTIVE

Test for an association between TTV and acute biopsy-proven alloreactivity after kidney transplantation.

MATERIAL & METHODS

Retrospective single center study analyzing 1010 consecutive renal allograft recipients: 113 patients were analyzed after applying inclusion and exclusion criteria.

TTV loads (in-house PCR) were quantified in the peripheral plasma.

For each of the 113 patients, one indication biopsy was included.

Primary endpoint: Acute biopsy-proven alloreactivity events, including antibody-mediated rejection (ABMR), T-cell mediated rejection (TCMR) and borderline changes suspicious of TCMR defined by Banff.

TTV loads from samples taken between month 4 to 12 post-transplantation were compared to subsequent allograft biopsy results.

RESULTS

Thirty-three (29%) biopsy samples showed features of acute alloreactivity (14 ABMR and 19 TCMR or borderline changes suspicious for acute TCMR).

Regarding type and amount of immunosuppression at the time of TTV quantification, tacrolimus level or estimated Glomerular Filtration Rate (eGFR), no difference was observed between patients with biopsy-proven alloreactivity and patients without rejection.

Patients with subsequent biopsy-proven alloreactivity had lower TTV loads (3.1×10^7 copies/mL) compared to patients without rejection ($2.3x10^8$ copies/mL) at a median of 43 days before the biopsy.

There is a linear association between TTV load and risk for alloreactivity in kidney transplant recipients: risk for alloreactivity decreased by 10% for every log increase in TTV load (95% CI 0.84-0.97).

To exclude rejection on the basis of TTV quantification, a TTV cut-off above 10^6 copies/mL showed:

- Sensitivity: 94%
- Specificity: 27%
- Positive predictive value (PPV): 76%
- Negative predictive value (NPV): 64%

Primary finding: Multivariable generalized linear modeling suggests an independent association between TTV load and kidney transplant recipients' alloreactivity.

Secondary finding: BK Virus-positive plasma samples (PCR) had a higher TTV load compared to BK negative samples: 3.1×10^9 copies/mL vs. 8.5×10^7 copies/mL.

"Our study provides evidence for the value of TTV quantification for risk stratification of biopsy-proven alloreactivity after kidney transplantation > 1 month before clinical diagnosis was made."

KEY FINDINGS

- TTV load is associated with biopsy-proven allograft rejection after kidney transplantation in month 4 to 12 post transplantation.
- TTV quantification could detect patients at risk for rejection more than 1 month before diagnosis by biopsy.
- A TTV load below 10^6 copies/mL poses a risk for subsequent rejection.

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JOURNAL OF CLINICAL MEDICINE
2020 FEB 6;9(2):440. DOI: 10.3390/JCM9020440

Torquetenovirus Serum Load and Long-Term Outcomes in Renal Transplant Recipients

E. Gore, A.W. Gomes-Neto, L. Wang, S.J.L. Bakker, H.G.M. Niesters, A.A.E. De Joode, E.A.M. Verschuuren, J. Westra, C. Van Leer-Buter

OBJECTIVE

Evaluate the use of TTV levels in the prediction of long-term outcomes after renal transplantation.

MATERIAL & METHODS

Retrospective study on 706 renal adult transplant recipients. After exclusion of patients with incomplete data, 666 cases were analyzed for the study.
All patients had a functional graft for at least one year before inclusion.
TTV viral load (TTV R-GENE®) done on serum samples.
Primary endpoint: all-cause mortality and death due to infectious causes.
Secondary endpoint: death due to graft failure (defined as return to dialysis or re-transplantation).

RESULTS

Patients were classified according to their TTV viral loads (undetectable / low / medium / high):

All-cause mortality

- Log_{10} TTV was significantly associated (P=0.02) with all-cause mortality in renal post transplantation. The risk of death increased by 12% per log_{10} TTV viral load increase (HR 1.12; 95% CI (1.02-1.23)).
- A cut-off TTV level of 3.65 log_{10} copies/mL highlighted patients with higher risk of death (75% specificity and 40% sensitivity).

Death due to infectious cause

- TTV viral load was significantly associated to the risk of death due to infection (HR 1.20 ; 95% CI (1.01–1.43)) (P=0.004)
- Patients with higher risk of death due to infections were identified as at risk when one TTV measurement was over 3.38 log_{10} copies/mL (55% sensitivity and 67% specificity).

Graft failure

- No significant difference in outcomes between the groups of patients,
- Results in contradiction with previous studies, probably due to bias regarding patient selection, all over 1 year post-transplant (the highest risk for acute rejection is within the first year post-transplant).

Time since transplantation and TTV

- Patients were redistributed according to the time since their transplantation: between 12 and 24 months and over 24 months.
- TTV levels in patients within 24 months from their transplantation were significantly higher than in patients over 24 months post-transplantation (P< 0.05).
- In patients over 24 months from transplantation, TTV measurement demonstrated a significant difference in survival between patients groups (P < 0.001), which is not the case with TTV measured within 24 months post-transplant.

"TTV-levels may be predictive of much longer-term outcomes [than] have been investigated thus far."

KEY FINDINGS

- Significant correlation between TTV levels and death due to all mortality causes and to infection.
- TTV level is a potential marker to evaluate long-term outcomes in renal transplantation recipients and is useful for their follow-up.
- Identification of potential cut-off of 3.65 log_{10} copies/mL to estimate long-term outcomes for renal transplantation recipients.
- Over 24 months, TTV levels significantly impact survival rate.
- TTV viral loads help detect patients with higher risk of death from all-cause mortality and infection.

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JOURNAL OF INFECTION
2019 JUL;79(1):56-60. DOI: 10.1016/J.JINF.2019.05.010. EPUB 2019 MAY 14

Torquetenovirus Viremia for Early Prediction of Graft Rejection after Kidney Transplantation

M. Solis, A. Velay, P. Gantner, J. Bausson , A. Filipputtu , R. Freitag , B. Moulin , S. Caillard , S. Fafi-Kremer

OBJECTIVE

Determine if TTV viremia may mirror the immunosuppression strength in order to better evaluate the risk of BK virus (BKV) replication and/or graft rejection in kidney transplant recipients (KTR).

MATERIAL & METHODS

Retrospective study on 66 adults with a kidney transplant.
Fifty patients with a BKV replication in urine in the first 24 months post-transplantation were included:

- 28 were also BKV-viremic, including 13 with biopsy-proven BK virus associated nephropathy (BKVAN)
- 16 without BKV replication during the first two years post-transplant were considered as the control group.

TTV viral load measured (TTV R-GENE®) in blood samples at day 0 (day of transplantation), M1, M3, M6, M12 and M24 after transplantation.
BKV viral load was measured (BK Virus R-GENE®) in urine and blood samples.

RESULTS

TTV kinetics post-transplantation on 86% of KTR (TTV positive) showed an increase of TTV levels from D0 (3.06 log_{10} copies/mL) to M3 (6.78 log_{10} copies/mL) and a decrease until M24 (4.55 log_{10} copies/mL) **(Figure 1)**.
86% of patients were TTV-positive at the time of transplantation, 96% during the follow-up.
TTV viral loads were lower in recipients of organ from living donors than from deceased donors particularly at M3 (−0.95 log_{10} copies/mL, P = 0.003) and M6 (−1.90 log_{10} copies/mL, P = 0.020).
Higher TTV load samples were associated with higher BKV load in patients with BKV replication in blood: when TTV loads increased by 0.2 log_{10}, BKV viremia increased by 1.5 log_{10} copies/mL (probability of 90%).
At M6, BKV-viremic patients showed higher TTV level compared to non-BKV-viremic patients with mean TTV loads of 6.93 vs. 5.47 log_{10} copies/mL respectively (P = 0.015)
Patients with subsequent graft rejection had significantly lower TTV loads compared to the group without rejection at D0 (−1.50 log_{10} copies/mL, P = 0.009).

At D0, when applying a TTV threshold of 3.4 log_{10} copies/mL: 39% observed graft rejection were under threshold at D0 (P=0.007; HR=7.30; 95% CI = (2.32–22.9); NPV=0.92; PPV=0.63) and 3% were above **(Figure 2A)**.
At M1, TTV load threshold of 4.2 log_{10} copies/mL: 48% observed graft rejection were under threshold at M1(P=0.001; HR=6.16; NPV=0.92; PPV=0.48) and 9% were above **(Figure 2B)**.

"While the development of BKV replication – essentially from donor origin in KTR – may rather depend on donor/recipient strain mismatch and serostatus, TTV loads might mirror more sustained and/or deeper immunosuppression leading to more intense BKV replication following transplantation."

KEY FINDINGS

- TTV load is a potential marker for immunosuppression strength to predict risk of BKV replication and kidney alloreactivity.
- Graft rejection may be predicted according to TTV loads at transplantation date and 1 month after transplantation:
  - At D0, a viral load threshold of 3.4 log_{10} copies/ml allowed prediction of graft rejection (HR = 7.30, NPV = 0.92, PPV = 0.63)
  - At M1, a viral load threshold of 4.2 log_{10} copies/ml also allowed prediction of graft rejection (HR = 6.16, NPV = 0.92, PPV = 0.48)
- High TTV loads correlate with high BKV viremia loads in patients showing BKV replication in blood.

Figure 1. TTV load dynamics after kidney transplantation.
Adapted from Solis M., *et al. Journal of Infection* 2019 Jul;79(1):56-60.

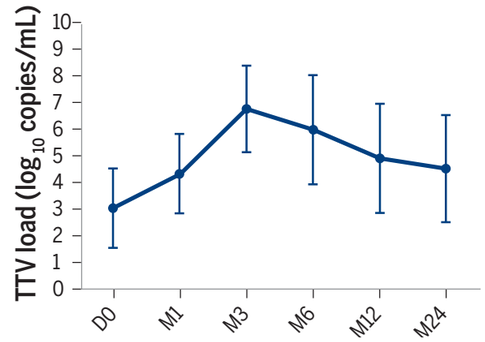
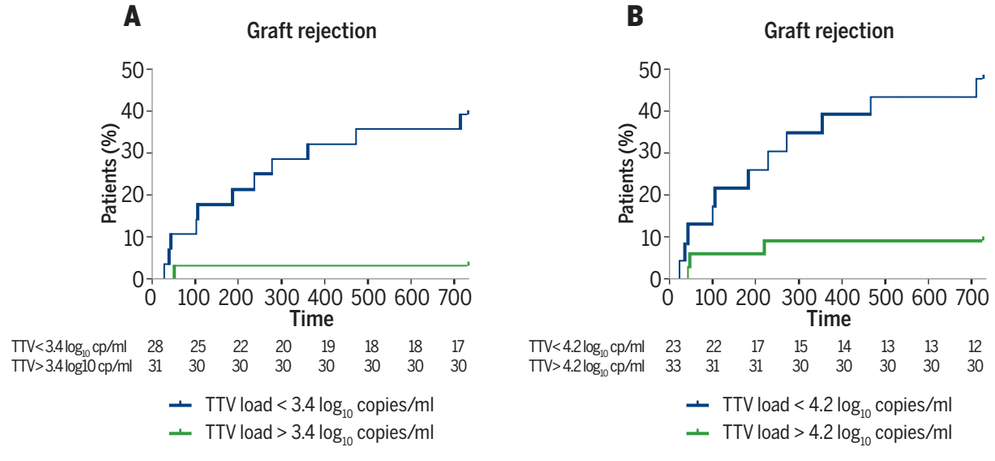


Figure 2. Prediction of graft rejection according to TTV load.
Adapted from Solis M., *et al. Journal of Infection* 2019 Jul;79(1):56-60.

Kaplan–Meier curves represent graft rejection cumulative incidence according to TTV viral load (log_{10} copies/ml) at the time of transplantation (panel A) and at M1 post transplantation (panel B).



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JOURNAL OF INFECTIOUS DISEASES
2018 SEP 8;218(8):1191-1199. DOI: 10.1093/INFDIS/JIY306

Quantification of Torque Teno Virus Viremia as a Prospective Biomarker for Infectious Disease in Kidney Allograft Recipients

R. Strassl, M. Schiemann, K. Doberer, I. Görzer, E. Puchhammer-Stöckl, F. Eskandary, Z. Kikic, G.A. Gualdoni, M.G. Vossen, S. Rasoul-Rockenschaub, H. Herkner, G.A. Böhmig, G. Bond

OBJECTIVE

Test for an association between TTV load and infectious disease after kidney transplant.

MATERIAL & METHODS

Prospective single center study including 169 consecutive kidney transplant recipient in the first year post-transplant.

TTV (in-house PCR), cytomegalovirus (CMV) and BK virus (BKV) quantification was performed every 3 months after transplantation.

Epstein-Barr virus (EBV) was performed in EBV IgG-negative patients every 3 months.

Primary endpoint: bacterial, fungal, or viral infection triggering a modification of immunosuppressive or antimicrobial/antiviral treatment.

TTV measurements taken after TTV load stabilization at the end of post-transplant month 3 were analyzed in the context of subsequent infection in the first year post transplantation.

RESULTS

Patient survival was 94% at the end of the follow-up period. The main cause of death was infection.

No difference in recipient age and sex, frequency of diabetes or other major comorbidities, intensity of induction therapy, and CMV prophylaxis was observed between patients with and without infections.

Patient with an infectious episode had higher TTV levels before the occurrence (median time of 77 days between TTV viral load and event) compared to measurements taken before an episode without infection ($4.2x10^8$ copies/mL vs. $2.9x10^7$ copies/mL).

Subgroup analyses were performed on:

- patients with severe infections (n=33) versus patients without infections: $6.4x10^8$ copies/mL vs. $2.9x10^7$ copies/mL.
- patients with bacterial infections vs. patients without infections: $4.4x10^8$ copies/mL vs. $2.9x10^7$ copies/mL.

Each TTV log increase led to an increase in the risk of infection by 23% (95% CI 1.04-1.45).

Multivariable generalized linear modeling suggests an independent association between TTV load and infection.

TTV levels above a threshold of $3.1x10^9$ copies/mL predict infection with a 90% sensitivity, a 20% specificity, a NPV of 92%, and a PPV of 17%.

"Taken together, our data suggest high levels of TTV reflect a state of intense immunosuppression after kidney transplantation, leading to an increased risk of infectious disease."

KEY FINDINGS

- Value of TTV loads to predict risk for infectious disease more than 2 months prior to occurrence in months 4 to 12 post kidney transplant.
- A TTV load above 10^9 copies/mL poses a high risk for subsequent infection.

CLICK TO ACCESS ARTICLE

TRANSPLANTATION
2017 FEBRUARY;101(2):360–367. DOI:10.1097/TP.0000000000001455

Torque Teno Virus Load – Inverse Association with Antibody Mediated Rejection after Kidney Transplantation

M. Schiemann, E. Puchhammer-Stöckl, F. Eskandary, P. Kohlbeck, S. Rasoul-Rockenschaub, A. Heilos, N. Kozakowski, I. Görzer, Ž. Kikić, H. Herkner, G. A. Böhmig, and G. Bond

OBJECTIVE

Test for an association between TTV load and late subclinical antibody-mediated rejection (ABMR) in kidney transplant recipients.

MATERIAL & METHODS

Cross-sectional single center study on 715 kidney transplant recipients that were screened for late ABMR (median 6.3 years post-transplant).

TTV viral load was assessed (in-house PCR) in plasma.

ABMR was diagnosed by biopsy and graded according to Banff.

RESULTS

Patients with late subclinical ABMR had a lower TTV load compared to patients without ABMR ($6.6x10^4$ copies/mL c/mL vs. $2.6x10^5$ copies/mL).

The risk for ABMR decreases by 0.91 per TTV log level increase (95% CI 0.87-0.96).

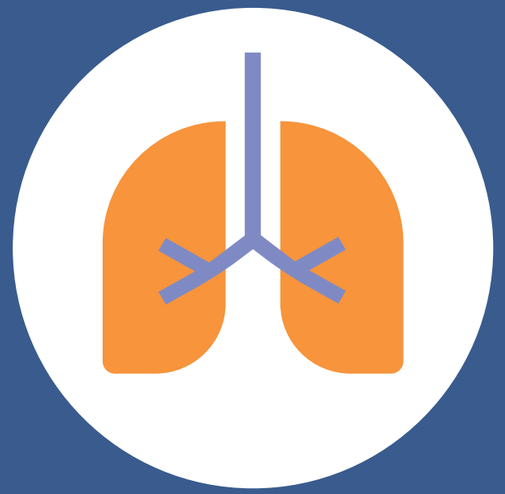
When focusing on the 86 patients subjected to biopsy, TTV load in ABMR-positive patients (n=40) was lower compared to ABMR-negative recipients (n=46; $4.5x10^5$ copies/mL vs. $6.6x10^4$ copies/mL).

Multivariable generalized linear modeling suggests an independent association between TTV load and kidney transplant recipients' alloreactivity.

"In conclusion, our data demonstrate an independent association between TTV load and late ABMR in recipients of a kidney transplant."

KEY FINDINGS

- Plasma TTV load in kidney transplant recipients was inversely associated with the occurrence of late subclinical ABMR.
- A TTV load below 10^5 copies/mL might pose a risk for the development of late ABMR.



LUNG TRANSPLANTATION

CELL
2013 NOVEMBER 21;155:1178–1187. DOI: 10.1016/J.CELL.2013.10.034

Temporal Response of the Human Virome to Immunosuppression and Antiviral Therapy

I. De Vlaminck, K. K. Khush, C. Strehl, B. Kohli, H. Luikart,N. F. Neff, J. Okamoto, T. M. Snyder, D. N. Cornfield, M. R. Nicolls, D. Weill, D. Bernstein, H. A. Valantine and S. R. Quake

OBJECTIVE

Observe the dynamics of human virome in post-transplant patients subjected to immunosuppression and antiviral therapies.

MATERIAL & METHODS

656 plasma samples were analyzed from 96 heart (adults and pediatrics) and lung (adults) recipients.

Only CMV-seropositive recipients (prior to transplantation) were given antiviral prophylaxis.

Microbiome-derived sequences were cleaned and mapped using Basic Local Alignment Search Tool (BLAST) against relevant reference databases.

RESULTS

Viruses were more abundantly represented (73%) than bacteria (25%) and fungi (2%).

Amongst viruses, *Anelloviridae* was the most prevalent family (68%) and was composed at 97% of *Alphatorquevirus* (TTV).

On a subgroup of adult heart and lung transplant recipients, viral composition varied largely upon immunosuppression and antiviral prophylaxis. Interestingly, TTV abundance was strongly impacted by immunosuppression strength, but not by antiviral therapy (valganciclovir) **(Figure 1)**.

The stronger the immunosuppression, the higher the TTV viral loads. Double-stranded (ds) DNA viruses (herpesvirales, caudovirales, adenovirales) were the most prevalent viruses of the virome (95%) in the first week post-transplant. Single-stranded (ss) DNA viruses (remaining 5%) are represented mainly by *Anelloviruses*.

By opposition, the relative abundance of ssDNA viruses increased rapidly after transplantation since they are not sensitive to antivirals, and take advantage of the immunosuppression regimen installed during the post-transplant management.

To assess whether *Anelloviruses* burden can be correlated with rejection, *Anelloviruses* quantification was performed on patients classified as rejecting (one biopsy-determined rejection, biopsy grade ≥ 2R/3A; 20 patients; 177 data points) and as non-rejecting (no diagnostics of moderate or severe graft rejection, biopsy grade ≤ 2R/3A; 40 patients; 285 data points).

Low *Anellovirus* loads were associated with an increase in the risk of rejection: inverse correlation between immune competence and risk of rejection **(Figure 2)**.

Anelloviruses load could help stratify patients as rejecting and non-rejecting patients (area under the curve = 0.72).

"The total burden of Anelloviruses identified in a transplant recipient's blood may serve as one such marker of the overall state of immunosuppression of the individual patient."

KEY FINDINGS

- Antiviral and immunosuppressive drugs impact the microbiome of transplant recipients in the post-transplant period.
- *Anelloviruses* are the most prevalent viruses very early in the post-transplant follow-up of transplant recipients. They are not sensitive to antivirals commonly used, but the immunosuppression strength positively impacts *Anellovirus* loads.
- *Anellovirus* loads are significantly inversely associated with the risk of developing graft rejection.

Figure 1. Mean virome composition for patients treated with the immunosuppressant tacrolimus (47 patients, 380 samples) as function of antiviral drug dose (valganciclovir) and concentration tacrolimus measured in blood.
Adapted from De Vlaminck I., *et al.* Cell 2013 Nov 21;155:1178-1187.

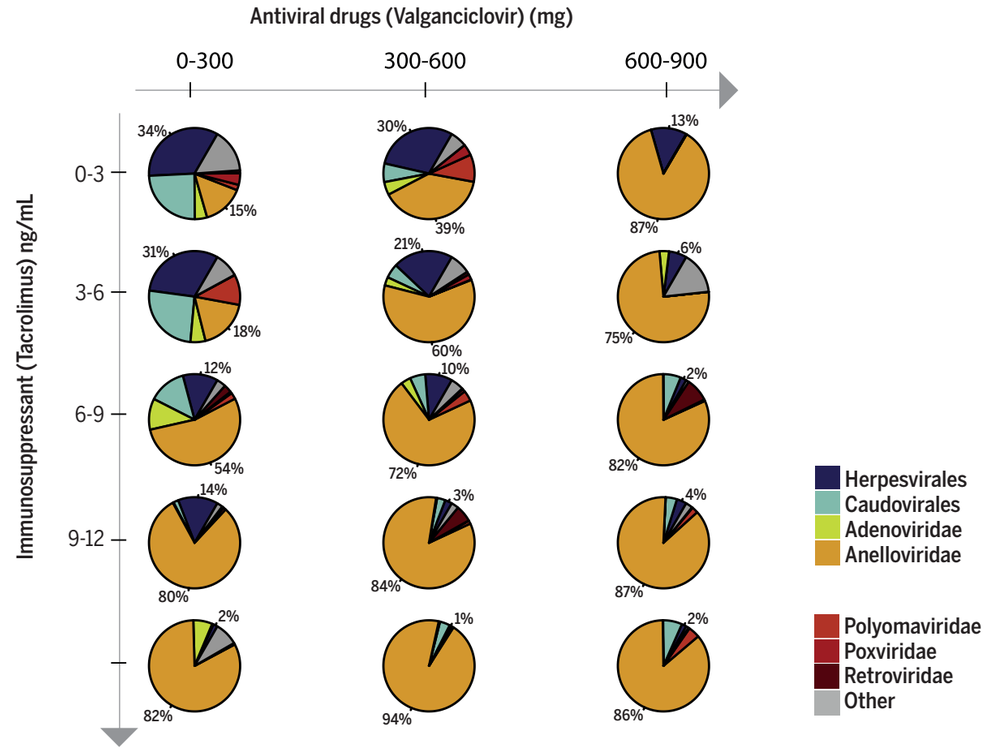
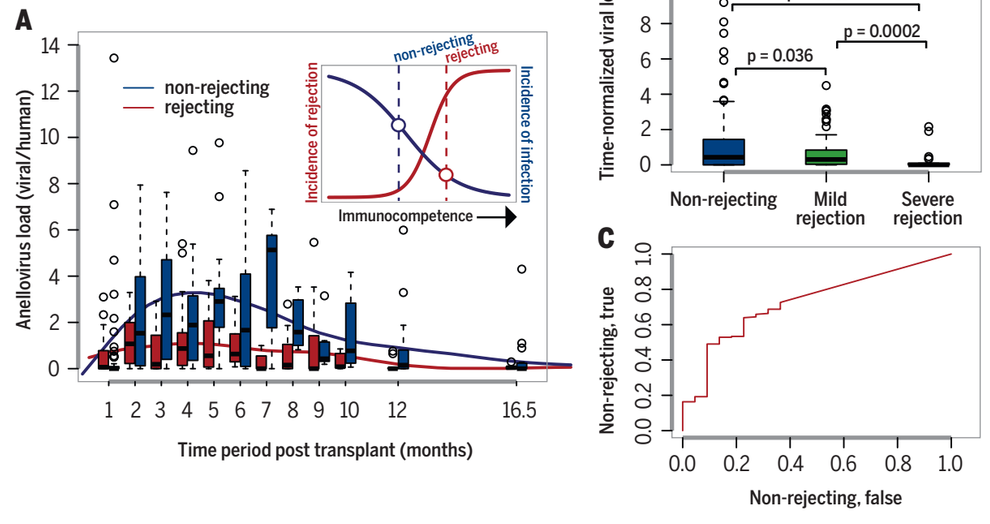


Figure 2. Lower *Anellovirus* burden in patients that suffer from graft rejection.
Adapted from De Vlaminck I., *et al.* Cell 2013 Nov 21;155:1178-1187.



JOURNAL OF HEART AND LUNG TRANSPLANTATION
2014;33(3):320-3. DOI:10.1016/J.HEALUN.2013.12.007

Plasma DNA Levels of Torque Teno Virus and Immunosuppression after Lung Transplantation.

I. Görzer, M. Haloschan, P. Jaksch, W. Klepetko, E. Puchhammer-Stöckl

OBJECTIVE

Observe immunosuppression intensity and occurrence of infection using TTV as a follow-up marker after lung transplantation.

MATERIAL & METHODS

Retrospective study on 31 patients followed-up for 2 years after transplantation: 465 plasma samples were collected.

Calcineurin inhibitors (CNIs) used in this study were tacrolimus (23 patients) and cyclosporine A (CsA, 8 patients).

Endpoints: document the kinetics of TTV loads after lung transplantation, relation between TTV, CNI-based immunosuppression and infectious complication.

RESULTS

TTV level kinetics during 2 years after lung transplantation:

- TTV viral loads peak 90 days after transplantation, remaining at a high steady state with slight decrease up to 2 years post transplant.

TTV level and CNI-based immunosuppression:

- Positive correlation of TTV level in plasma and tacrolimus doses (r=0.68; *P*<0.001) and similar observation for CsA (r=0.89; *P*=0.012).
- Between day 60 and 630, patients with CsA treatments showed significantly lower TTV levels than tacrolimus-treated patients **(Figure 1)**.

TTV level and infectious episode:

For 13/20 patients with complications, high TTV levels were observed from 28 to 76 days before the infectious episode. TTV levels were higher than the highest levels of the control group (*P*=0.045).

A cutoff of 9.3 $\log_{10}$ copies/mL was shown to predict the development of infection with a 53.85% sensitivity and a 90.92% specificity: patients with TTV viral load above 9.3 $\log_{10}$ copies/mL have a significantly higher risk for microbial infections than patients with lower TTV viral load (87.5% vs 37.5% respectively; *P*=0.033).

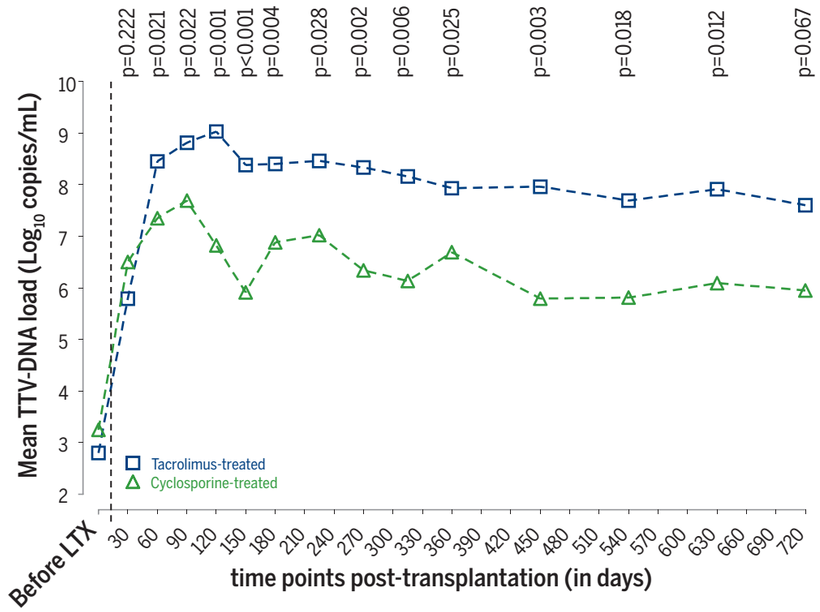
"In conclusion, our findings provide evidence that the plasma DNA level of TTV, a virus that is persistently and asymptomatically replicating in humans, and whose level of replication is subject to human immune response, is related to the state of immunosuppression after lung transplantation."

KEY FINDINGS

- TTV viral load is related to the net immunosuppression state in lung transplant recipients.
- A threshold of TTV viral load above 9.3 $\log_{10}$ copies/mL might help identify patients at risk for infection after lung transplantation.

Figure 1. Mean plasma TTV DNA kinetics of 23 patients with tacrolimus treatment and 8 with cyclosporin A (CsA) treatment are shown. At each time point after transplantation, TTV DNA plasma loads between tacrolimus-treated and CsA-treated patients were compared by Mann-Whitney U test.

Adapted from Görzer I,. *et al.* Journal of Heart and Lung Transplantation 2014;33(3):320-3.



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JOURNAL OF HEART AND LUNG TRANSPLANTATION
2017 MAR;36(3):366-368. DOI:10.1016/J.HEALUN.2016.10.011

Association between Plasma Torque Teno Virus Level and Chronic Lung Allograft Dysfunction after Lung Transplantation

I. Gorzer , P. Jaksch , R. Strassl, W. Klepetko, E. Puchhammer-Stockl

OBJECTIVE

Evaluate a potential correlation between TTV viral load and chronic lung allograft dysfunction (CLAD) after lung transplantation.

MATERIAL & METHODS

Case-control study on 20 lung recipients who developed CLAD within 3 years post-transplant.

The control group was formed of 27 lung transplant recipients without any signs of CLAD chosen according to their age to match the case group.

Plasma TTV load was quantified by in-house real-time PCR.

RESULTS

At CLAD onset, the plasma TTV viral loads of the control group were significantly lower than the TTV loads of the CLAD group (*P*= 0.0047).

A cut-off of 7 log_{10} copies/mL showed a 65% sensitivity, 81.5% specificity, positive predictive value (PPV) 72% and negative predictive value (NPV) 76% to distinguish patients with CLAD from control patients: patients with a TTV viral load < 7 log_{10} copies/mL were shown to be more prone to develop CLAD.

TTV viral loads in samples taken 50 days +/- 20 days prior to CLAD onset were significantly lower (*P*=0.0337) than in the respective controls. A cut-off of 7 log_{10} copies/mL was again shown to be associated with CLAD occurrence with a 64.3% sensitivity, 86.7% specificity, PPV 82% and NPV 72%.

To assess if TTV load can predict CLAD onset even earlier, TTV viral load was assessed in patients samples taken within a period of 160 +/- 50 days before CLAD occurrence. Again, the TTV viral load was significantly lower (*P*= 0.0195) in patients developing CLAD.

"TTV monitoring in the follow-up after lung transplantation may be helpful in identifying patients who are at particular risk for organ rejection."

KEY FINDINGS

- Although this was a pilot study, with a limited number of patients included, the results demonstrate the potential of TTV to predict chronic lung allograft dysfunction, up to 5 months prior to CLAD onset.
- A cut-off of 7.0 log_{10} copies/mL has been found to identify patients at high risk of developing CLAD.

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JOURNAL OF INFECTIOUS DISEASES
2018 NOV 5;218(12):1922-1928. DOI: 10.1093/INFDIS/JIY452

Torque Teno Virus as a Novel Biomarker targeting the Efficacy of Immunosuppression after Lung Transplantation

P. Jaksch, M. Kundi, I. Görzer, G. Muraközy, C. Lambers, A. Benazzo, K. Hoetzenecker, W. Klepetko, E. Puchhammer-Stöckl

OBJECTIVE

Observe the potential correlation between TTV viral load and development of acute and chronic rejection, as well as infectious complications.

Translate the immunosuppression profile intensity to predict rejection or infection.

MATERIAL & METHODS

Prospective study on 143 lung recipients presenting a minimal survival of 6 months and minimum of 10 blood samples collected. A total of 3020 samples were analyzed.

Quantification of TTV in blood samples was performed at intervals between every two weeks and 2 months.

All events such as death, infection, chronic lung allograft dysfunction (CLAD) or acute cellular rejection (ACR) were documented.

RESULTS

Among the 143 lung transplant recipients, 28 were hospitalized for infection (viral, bacterial, fungal); 22 had CLAD; 11 had ACR; 3 had retransplantation and 24 died.

Above a TTV load of 9.5 log_{10} copies/mL observed in a cumulative 3-month window, the risk of developing an infection increased to >40% and no infectious events were observed in patients with a TTV viral load below 7 log_{10} copies/mL.

For ACR, the lower levels of TTV in 3-month windows could predict the occurrence of ACR. For each TTV log_{10} increase, the risk of ACR occurrence decreased by around 50%. ACR events were more frequent for TTV under 7 log_{10} copies/mL.

For CLAD, the only significant predictive variable found was the minimal TTV viral load in the 3-month window. Higher TTV levels translated to decreased risk in CLAD occurrence: for each log_{10} copies/mL increase, the risk was reduced to about 70%.

Analyses of potential cutoffs by receiver operating characteristic (ROC) found:

- upper threshold to predict infection risk was **9.2 log_{10} copies/mL** of TTV loads
  - sensitivity: 87%; specificity: 71%
- lower threshold to predict CLAD risk was **8.1 log_{10} copies/mL** TTV loads
  - sensitivity: 95%; specificity: 55%

"In conclusion, the present study provides evidence that Alphatorquevirus load may serve as a useful marker to assess the level of immunosuppression in lung transplant recipients and to predict clinical complications."

KEY FINDINGS

- TTV viral load correlated with the risk of developing infections, acute cellular rejection and chronic lung allograft dysfunction.
- Definition of thresholds to significantly lower risk of infection and CLAD.

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PEDIATRIC TRANSPLANTATION
2018;22:E13069. DOI.ORG/10.1111/PETR.13069

Anellovirus Loads are associated with Outcomes in Pediatric Lung Transplantation

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OBJECTIVE

Observe the association between *Anelloviruses* (*Alpha-* and *Betatorqueviruses*) loads and short/long-term effects after lung transplantation in the pediatric population.

MATERIAL & METHODS

61 patients aged less than 18 years old were enrolled, but only 57, who had sufficient blood collection at 2 weeks, 6 weeks and 6 months post-transplant, were included in this study.

Standardized immunosuppressant and antiviral prophylaxis protocols were applied.

Alphatorquevirus (*torque teno viruses*) and *Betatorquevirus* (*torque teno mini viruses*) loads were measured with real time PCR at week 2, week 6 and month 6 post-transplant.

Outcomes at short term (primary graft dysfunction (PGD), acute cellular rejection (ACR)) and long term (death, chronic lung allograft dysfunction (CLAD) and retransplant) were observed.

RESULTS

ACR was reported for more than 1/3 of patients within 3 months post-transplant and 1/3 had long-term outcomes (death, bronchiolitis obliterans/ bronchiolitis obliterans syndrome (BO/BOS), retransplant) within 2 years post-transplant. Paired levels of *Alphatorquevirus* and *Betatorquevirus* were not statistically associated with these results ($P>0.05$).

***Anellovirus* levels at 2 weeks after transplantation:**

- Patients with TTV levels below the median have a significantly higher risk of developing ACR within 3 months ($P=0.013$).
- Low levels of TTV at week 2 post-transplant had 17 times the odds of experiencing an ACR within 3 months ($P=0.021$).

***Anellovirus* levels at 6 weeks after transplantation:**

- Association between *Betatorquevirus* loads and death within 2 years post-transplant ($P=0.047$).
- Low *Betatorquevirus* levels were an indicator of death within 2 years post-transplant ($P=0.022$) : 100% (5/5) of deceased patients within 2 years had *Betatorquevirus* levels below the median.

***Anellovirus* levels at 6 weeks after transplantation:**

- Chronic rejection, retransplantation and deaths were significantly associated with low *Betatorquevirus* levels ($P=0.017$).

"... we propose that Anellovirus has utility not only for estimating immune suppression, but also for predicting clinical outcomes. [...]. Given the apparent utility of early post- transplant Anellovirus levels, pretransplant levels should be measured in a future study as it is possible they could be used to stratify recipient risk."

KEY FINDINGS

- *Alphatorquevirus* load (i.e. *torque teno viruses*) at week 2 post-transplant has a predictive value to identify patients with higher risk of short-term adverse outcomes, such as acute cellular rejection.
- *Betatorquevirus* load (i.e. *torque teno mini viruses*) at week 6 and month 6 post-transplant has a predictive value to identify patients with higher risks of long-term adverse outcomes (death, BO/BOS and retransplant).

LIVER TRANSPLANTATION

TRANSPLANTATION JOURNAL
2017:101(7):E219-E221. DOI: 10.1097/TP.0000000000001723

Torque Teno Virus Load and Acute Rejection After Orthotopic Liver Transplantation

F. Simonetta, A. Pradier, S. Masouridi-Levrat, C. Van Delden, E. Giostra, I. Morard, N. Mueller, B. Muellhaupt, P. V. Valli, N. Semmo, J. Seebach, Y. Chalandon, L. Kaiser, E. Roosnek, and Swiss Transplant Cohort Study (STCS)

OBJECTIVE

Determine the correlation between TTV viral load and the occurrence of biopsy-proven acute cellular rejection (ACR) after orthotopic liver transplantation (OLT).

MATERIAL & METHODS

Thirty-nine patients were included and their TTV load was measured at the time of transplantation. Among them, 19 patients with sufficient serum samples were additionally tested for TTV viral loads at month 6 and month 12 after transplantation.

A control group was composed of 74 healthy subjects.

Acute cell rejection events (score ≥ 2 or ≥3 with significant necrosis) were documented during the first year post-transplant.

RESULTS

TTV viral loads were significantly higher in patients with orthotopic liver transplantation than in healthy subjects.

TTV levels were significantly different between each subgroup analysis.

A significantly lower 1-year biopsy-proven acute rejection cumulative incidence was observed in OLT patients with detectable TTV viral load at the time of transplantation (P=0.042).

KEY FINDINGS

- Higher TTV loads in liver transplant patients than in controls.
- Non-detectable TTV loads were correlated to patients at higher risk of developing ACR.

LIVER TRANSPLANTATION
2019;25(2):302-310. DOI: 10.1002/LT.25374

Torque Teno Virus Is Associated With the State of Immune Suppression Early After Liver Transplantation

P. Ruiz, M. Martínez-Picola, M. Santana, J. Muñoz, S. Pérez-Del-Pulgar, G. Koutsoudakis, L. Sastre, J. Colmenero, G. Crespo and M. Navasa

OBJECTIVES

Assess the correlation between TTV viral load and the occurrence of acute cellular rejection (ACR) and CMV infection in liver recipients, during the first year post-transplant.

Document TTV load in tolerant and long-term liver recipients.

MATERIAL & METHODS

Prospective follow-up was performed on 63 liver transplant recipients during 1 year post-transplant.

TTV quantification was done before transplantation, at week 1, and months 1, 3, 6 and 12 in post-transplantation.

TTV DNA levels were measured by PCR and both acute cellular rejection (ACR) and cytomegalovirus infection (CMV-infection) were reported.

Long-term assessment was performed on 3 different groups:

- 10 long-term liver recipients treated with tacrolimus
- 10 tolerant recipients, i.e. without immunosuppressive drugs for more than 12 months but maintaining normal graft function and histology
- 10 healthy controls

RESULTS

TTV viral loads reached a peak at 3 months post-transplant and then decreased until the last time point, i.e. 12 months, post-transplant.

When considering only clinical episodes of rejection, patients with ACR presented lower TTV loads (4.41 $\log_{10}$ copies/mL versus 5.95 $\log_{10}$ copies/mL; P=0.002) during ACR.

During episodes of CMV infection, TTV levels were reported to be significantly higher compared to other time points (6.59 $\log_{10}$ copies/mL versus 5.79 $\log_{10}$ copies/mL; P=0.009)

During CMV disease, TTV viral load was significantly higher in patients with CMV disease (8.20 $\log_{10}$ copies/mL versus 5.85 $\log_{10}$ copies/mL; P=0.005)

Long-term assessment of the different patients' profiles did not show any significant differences regarding TTV viral load.

TTV viral loads at the earliest available time points (before transplantation, at week 1 or month 1) were assessed as a potential predictor for ACR or CMV infection occurrence. The predictive ability was found to be low.

Table 1. TTV cut-offs and associated risk predicted.

Adapted from Ruiz P. *et al. Liver Transplantation* 2019;25(2):302-310.

TTV cut-off	Risk predicted	Specificity	Sensitivity	NPV*	PPV*
> 7.50 $\log_{10}$ copies/mL	CMV infection	84%	80%	100%	38%
> 4.75 $\log_{10}$ copies/mL	ACR	77%	100%	98%	30%

* Negative Predictive Value ** Positive Predictive Value

"... plasma TTV DNA levels are associated with immune-related events after LT and could constitute a potential biomarker of the state of IS during the first months after transplant."

KEY FINDINGS

- Potential value of TTV as a biomarker for immune-related events (ACR and CMV infections) in the first year post liver transplant.
- Association of TTV viral loads with occurrence of ACR and infection events.
- Establishment of predictive TTV values for acute cellular rejection (< 4.75 $\log_{10}$ copies/mL) and CMV infection (>7.50 $\log_{10}$ copies/mL).

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NOTES

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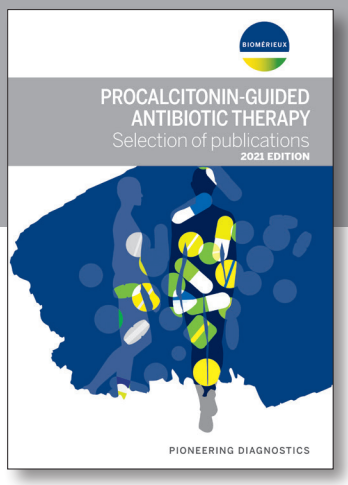

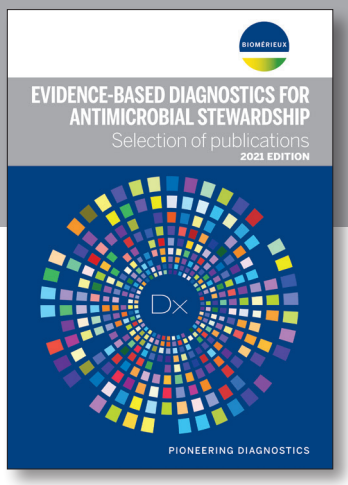

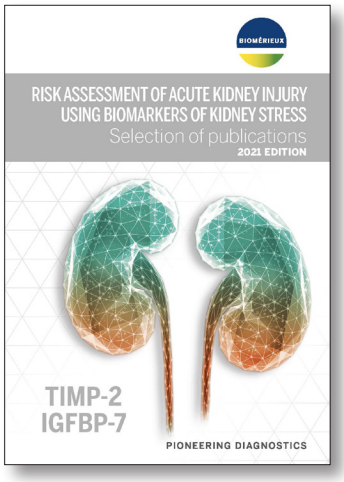

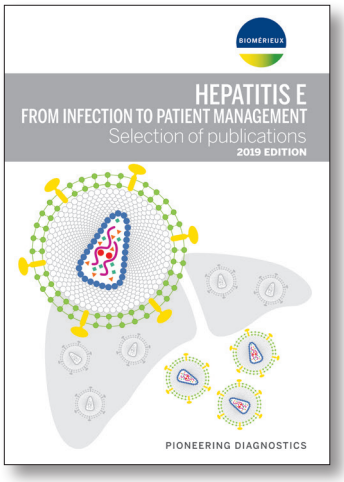

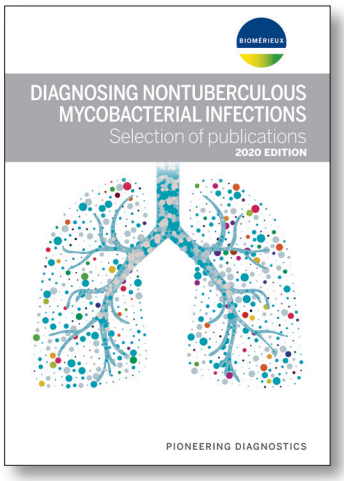

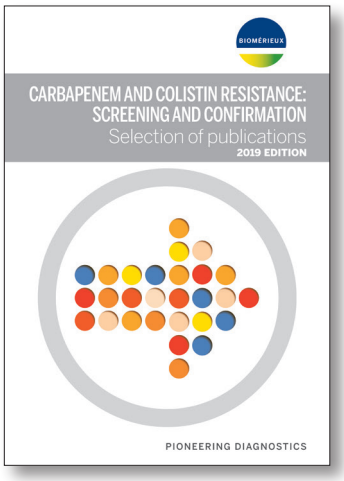

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